A single bout of exercise induces β-adrenergic desensitization in human adipose tissue

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Marion-Latard, Fabrice, Isabelle De Glisezinski, Francois Crampes, Michel Berlan, Jean Galitzky, Hana Suljkovichova, Daniel Riviere, and Vladimir Stich. A single bout of exercise induces β-adrenergic desensitization in human adipose tissue. Am J Physiol Regulatory Integrative Comp Physiol 280: R166–R173, 2001.—This study was designed to assess whether physiological activation of the sympathetic nervous system induced by exercise changes adipose tissue responsiveness to catecholamines in humans. Lipid mobilization in abdominal subcutaneous adipose tissue was studied with the use of a microdialysis method in 11 nontrained men (age: 22.3 ± 1.5 yr; body mass index: 23.0 ± 1.6). Adipose tissue adrenergic sensitivity was explored with norepinephrine, dobutamine (β1-agonist), or terbutaline (β2-agonist) perfused during 30 min through probes before and after 60-min exercise (50% of the maximal aerobic power). The increase in extracellular glycerol concentration during infusion was significantly lower after the exercise when compared with the increase observed before the exercise (P < 0.05, P < 0.02, and P < 0.01, respectively, for norepinephrine, dobutamine, and terbutaline). In a control experiment realized without exercise, no difference in norepinephrine-induced glycerol increase between the two infusions was observed. To assess the involvement of catecholamines in the blunted β-adrenergic-induced lipolytic response after exercise, adipose tissue adrenergic sensitivity was explored with two 60-min infusions of norepinephrine or epinephrine separated by a 60-min interval. With both catecholamines, the increase in glycerol was significantly lower during the second infusion (P < 0.05). The findings suggest that aerobic exercise, which increased adrenergic activity, induces a desensitization in β1- and β2-adrenergic lipolytic pathways in human subcutaneous adipose tissue.

Physical activity promotes activation of the SNS and an increase in circulating catecholamine levels in the blood (19). During exercise, glycerol output from subcutaneous adipose tissue (3, 15, 35) and glycerol plasma concentrations (25) are increased. At present, there are no data that demonstrate whether desensitization occurs after a physiological activation of the SNS induced by exercise in humans.

This study was designed to assess whether exercise-induced activation of the sympathoadrenal system had a functional impact on in situ human adipose tissue responsiveness to catecholamines and selective β-AR agonists. The study was carried out with the use of the microdialysis technique (1, 5, 35). This method allows vitro, the β-AR was shown to be regulated by β-agonist agents; when target cells are in the presence of β-AR agonists, the receptors become desensitized within a few minutes. This phenomenon also occurs in human fat cells, and in vitro studies have shown that isolated adipocytes exposed to β-AR agonists show a decrease in functional coupling between β-AR and adenylate cyclase (10, 21). Recent studies have demonstrated that desensitization of the β-adrenergic response in rat adipocytes involves various receptor subtypes and cAMP phosphodiesterase (7). In vivo desensitization of the β-adrenergic response was shown in white adipocytes of various species (12, 22), and a differential regulation of β1- and β2-AR has been described (9, 32). In humans, during prolonged local epinephrine infusion, concentrations of interstitial glycerol tend to fall after a transient increase (2). Stallknecht et al. (34) showed that prior exposure of adipose tissue to epinephrine induced a decrease of a subsequent lipolytic effect of the hormone during a second intravenous epinephrine infusion. These findings suggest that catecholamines induce desensitization of adipose tissue lipolysis in humans.

The biological functions of the sympathetic nervous system (SNS) are mediated by the activation of α- and β-adrenergic receptors (AR) by catecholamines. In
measurement of extracellular (EC) concentrations of various substances that diffuse from the interstitium of adipose tissue into the infusion fluid. It also enables administration of various pharmacologically active substances into the tissue through the microdialysis probe. In this study, norepinephrine, dobutamine (β1-AR agonist), and terbutaline (β2-AR agonist) through the subcutaneous adipose tissue were infused before and after physical exercise to assess the exercise-induced desensitization. In control experiments, repeated infusions of epinephrine and norepinephrine, without any exercise in between, were performed.

METHODS

Subjects

Eleven nontrained men were selected for this study. Their mean age was 22.3 ± 1.5 yr, and their body mass index was 23.0 ± 1.6 kg/m². All subjects had a stable body weight for at least 3 mo before the beginning of the study, and all were drug free. The subjects had given their informed consent before the study, which was performed according to the declaration of Helsinki and approved by the Ethical Committee of the University Hospital.

Microdialysis Method

The subjects were investigated at 0800 after an overnight fast. They were placed in a semirecumbent position. Two microdialysis probes (Carnegie Medecin, Stockholm, Sweden) of 20 × 0.5 mm and 20,000-MW cutoff were inserted percutaneously after epidermal anesthesia (200 μl 1% lidocaine; Roger-Bellon, Neuilly-s-Seine, France) into the abdominal subcutaneous adipose tissue at a distance of 10 cm from the umbilicus. The probes were separated by 5 cm and were connected to a microinjection pump (Harvard apparatus, S.A.R.L., Les Ulis, France). They were perfused with a sterile Ringer solution [(in mM) 154 sodium, 4 potassium, 2.5 calcium, and 160 chloride] supplemented with ethanol (1.7 g/l). Ethanol was added to the perfusate to estimate changes in the adipose blood flow, as previously described (4, 5). After a 30-min equilibration period, dialysate was collected for 30 min at a flow rate of 0.5 μl/min. Then, the probes were perfused at 2.5 μl/min for the remaining experimental period. The estimated glycerol EC concentrations were calculated by plotting (after log transformation) the concentration of glycerol in the dialysate measured at 0.5 and 2.5 μl/min against the infusion rates. The values given in RESULTS fit with previous determinations in lean subjects (4, 5, 24). According to the experimental schedule, various catecholamines or selective β1- or β2-AR agonists (dobutamine and terbutaline, respectively) were added to the perfusate. During the whole experiment, dialysate was collected every 15 min, except during the 30-min adrenergic agent infusions where dialysate was collected every 10 min. The fractions were kept on ice; glycerol and ethanol analysis was performed in each fraction collected. The changes in nutritive blood flow were assessed using the ethanol outflow/inflow ratio measurement, as previously described (17, 20).

Experimental Schedule

First experiment. After calibration of the two probes, six subjects were infused for 30 min with Ringer solution containing either 10 μM of norepinephrine (Sterling-Wintrop, Gentilly, France), 100 μM of the selective β1-AR agonist dobutamine (Dobutrex, Lilly France SA, France), or 100 μM of the selective β2-AR agonist terbutaline (Bricanyl, Astra, France). After that, the probes were infused for 60 min with the Ringer solution. Then, the subjects performed a cycling exercise for 60 min. After that, they were allowed to rest in the semirecumbent position for 60 min. The probes were infused again for 30 min with Ringer solution containing either 10 μM of norepinephrine, 100 μM of the selective dobutamine, or 100 μM of terbutaline. Finally, the probes were infused for 60 min with the Ringer solution while the subjects maintained the semirecumbent position (Figs. 1A and 2, A and B). A control investigation was carried out on the same subjects, with similar timing and norepinephrine infusion, but without the exercise period (Fig. 1B).

Fig. 1. A: effect of norepinephrine (Nor) on the interstitial glycerol levels (●) and the ethanol ratio (○) in subcutaneous adipose tissue before and after 60-min exercise. Nor was perfused for 30 min, 90 min before exercise, and 60 min after the cessation of the exercise. Values are means ± SE. The areas under the curves of the Nor-induced increase in interstitial glycerol concentrations were significantly lower after exercise (P < 0.05). B: effect of Nor on the interstitial glycerol levels (●) and the ethanol ratio (○) in subcutaneous adipose tissue. First Nor infusion was performed for 30 min, then a second one was performed after 180 min. Values are means ± SE. The areas under the curves of Nor-induced increase in interstitial glycerol concentrations were not significantly different for the 2 Nor infusions.
Second experiment. After calibration of the two probes, five subjects were infused for 60 min with Ringer solution containing either 10 μM norepinephrine or 10 μM epinephrine (epinephrine, Aguettant, Lyon, France). After that, the probes were infused with the Ringer solution during 60 min. The probes were infused again for 60 min with Ringer solution containing either 10 μM norepinephrine or 10 μM epinephrine. Finally, the probes were infused for 60 min with the Ringer solution. In this second experiment, dialysate was collected every 15 min (Fig. 3, A and B).

Exercise

In the first experiment, the subjects performed an exercise for 60 min on a cycle ergometer (Ergo-metrics 800S Ergo-line, Jaeger, Germany). The imposed power corresponded to 50% of the maximal aerobic power. The heart rate was continuously monitored with a Polar Accurex Plus cardiometer (Monitor, France) during the exercise. In all the protocols, the exercise bout was started 60 min after the end of infusion with adrenergic agents so that the increased EC levels after the pharmacological stimulation were back at baseline.

Blood samples were drawn from an indwelling polyethylene catheter introduced into an antecubital vein 10 min before the exercise and every 15 min during the exercise and recovery for glycerol, nonesterified acids, lactate, glucose, insulin, and catecholamine assays. Blood was immediately centrifuged at 0°C, and the plasma was stored at −80°C until analysis.

Fig. 2. A: effect of the selective β1-adrenoceptor agonist dobutamine (Dob) on the interstitial glycerol levels (●) and the ethanol ratio (○) in subcutaneous adipose tissue before and after 60 min exercise. Dob was perfused for 30 min, 90 min before exercise, and 60 min after the cessation of the exercise. Values are means ± SE. The areas under the curves of Dob-induced increase in interstitial glycerol concentrations were significantly lower after exercise (P < 0.02). B: effect of the selective β2-adrenoceptor agonist terbutaline (Ter) on the interstitial glycerol levels (●) and the ethanol ratio (○) in subcutaneous adipose tissue before and after 60 min exercise. Ter was perfused for 30 min, 90 min before exercise, and 60 min after the cessation of the exercise. Values are means ± SE. The areas under the curves of Ter-induced increase in interstitial glycerol concentrations were significantly lower after exercise (P < 0.01).

Fig. 3. A: effect of Nor on the interstitial glycerol levels (●) and the ethanol ratio (○) in subcutaneous adipose tissue. First Nor infusion was performed for 60 min, then a second 60-min infusion was performed after 1 h. Values are means ± SE. The area under the curve of the Nor-induced increase in interstitial glycerol concentrations was significantly lower for the second Nor infusion (P < 0.05). B: effect of epinephrine (Epi) on the interstitial glycerol levels (●) and the ethanol ratio (○) in subcutaneous adipose tissue. First Epi infusion was performed for 60 min, then a second 60-min infusion was performed after 1 h. Values are means ± SE. The area under the curve of the Epi-induced increase in interstitial glycerol concentration was significantly lower for the second Epi infusion (P < 0.05).
EXERCISE-INDUCED DESENSITIZATION IN ADIPOSE TISSUE

Table 1. Effect of exercise on plasma glycerol, NEFA, glucose, insulin, catecholamine, and lactate levels

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>30-min Exercise</th>
<th>60-min Exercise</th>
<th>60-min Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol, µM</td>
<td>68.8 ± 7.9</td>
<td>117.9 ± 16.3*</td>
<td>126.6 ± 21.4*</td>
<td>92.4 ± 8.2</td>
</tr>
<tr>
<td>NEFA, µM</td>
<td>287.6 ± 54.7</td>
<td>258.1 ± 62.3</td>
<td>303.7 ± 64.6</td>
<td>372.7 ± 43.3</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>4.78 ± 0.17</td>
<td>4.66 ± 0.21</td>
<td>4.56 ± 0.21</td>
<td>4.55 ± 0.14</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>5.60 ± 0.65</td>
<td>4.77 ± 0.39</td>
<td>3.61 ± 0.33*</td>
<td>3.96 ± 0.22</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>34.8 ± 6.7</td>
<td>87.5 ± 7.1*</td>
<td>143.5 ± 16.1*</td>
<td>65.4 ± 16.2</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>172.5 ± 27.1</td>
<td>902.3 ± 104.9*</td>
<td>1,061.5 ± 104.9*</td>
<td>233.9 ± 29.1</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>0.9 ± 0.1</td>
<td>1.5 ± 0.3*</td>
<td>1.3 ± 0.1</td>
<td>1.1 ± 0.2</td>
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</table>

Values are means ± SE (n = 6). *P < 0.05 exercise vs. rest. NEFA, nonesterified fatty acids.

Analytic Methods

For each investigation, two probes were inserted, and the glycerol and ethanol results were the mean of the values of the two probes. Glycerol in dialysate (10 µl) and in plasma (20 µl) was analyzed with an ultrasensitive radiometric method (8); the intra- and interassay variabilities were 5 and 9.2%, respectively. Ethanol in dialysate and in perfusate (5 µl) was determined with an enzymatic method (6); the intra- and interassay variabilities were 3 and 4.5%, respectively. Plasma nonesterified fatty acids (NEFA) and lactate were determined with an enzymatic procedure (Wako, Unipath, Illkirch, France), respectively. Plasma glucose was determined with a glucose oxidase technique (Biotrol, Merck-Clevenot, Illkirch, France). Plasma immunoreactive insulin was measured with the use of an RIA kit from ERIA Diagnostics Pasteur (Marnes-la-Coquette, France). Plasma glucose and norepinephrine were assayed in 1-ml aliquots of plasma by high-pressure liquid chromatography with the use of an automated analyzer (YSI 27, Bioblock Scientific, Illkirch, France). Plasma epinephrine and norepinephrine were assayed in plasma by a glucose oxidase technique (Biotrol, Merck-Clevenot, Illkirch, France), respectively. Plasma glucose was determined with a glucose oxidase technique (Biotrol, Merck-Clevenot, Illkirch, France). Plasma immunoreactive insulin was measured with the use of an RIA kit from ERIA Diagnostics Pasteur (Marnes-la-Coquette, France). Plasma epinephrine and norepinephrine were assayed in 1-ml aliquots of plasma by high-pressure liquid chromatography with the use of an enzyme method (26); the detection limit was 20 pg/sample, day-to-day variability was 4%, and within-run variability was 3%.

Statistical Analysis

All the values are expressed as means ± SE. The responses to perfusions were analyzed with the use of a paired t-test. During perfusions, the EC glycerol concentration response and ethanol ratio value were calculated as the total integrated changes over baseline values [area under curve (AUC)] from time (t) = 15 to t = 45 min and t = 225 to t = 255 min for the first experiment and from t = 15 to t = 75 min and t = 135 to t = 195 min for the second experiment]. AUC was calculated with the use of a trapezoidal method. Significant values are quoted in Tables 1 and 2. P < 0.05 was considered statistically significant.

RESULTS

Effect of Exercise on EC Glycerol Concentrations and Ethanol Ratio Induced by Local Norepinephrine Infusion in Adipose Tissue

The first infusion of norepinephrine that was started 90 min before the exercise induced a significant increase in EC glycerol by 319 ± 93 µM after a 30-min infusion (Fig. 1A). Norepinephrine infusion promoted a vasoconstriction as shown by the observed increase (+5.8 ± 1.9%) in the ethanol outflow/inflow ratio. At the beginning of the exercise bout (60 min after the end of infusion), the EC glycerol concentration was back at baseline. Moreover, the baseline ethanol outflow/inflow ratio was not significantly different to that before the norepinephrine infusion. During the exercise, significant increases in EC and plasma glycerol, plasma norepinephrine, and epinephrine levels were observed after 30 and 60 min of exercise (Table 1). Plasma NEFA and glucose levels were unchanged, and a significant decrease in insulin plasma level was observed after 60 min of exercise (Table 1). Plasma lactate levels were not significantly increased at the end of exercise. All the above-mentioned parameters as well as EC glycerol concentration and ethanol outflow/inflow ratio returned to levels not different from baseline after 60 min of recovery. The second infusion of norepinephrine, starting 60 min after the end of the exercise, induced an increase in EC glycerol concentration of 149 ± 12 µM. The overall response, when evaluated from the AUC, was significantly lower than during the first infusion (Table 2).

In a control experiment, two 30-min norepinephrine infusions separated by 180 min were carried out, but no exercise was performed by the subjects. In these conditions, the increase in EC glycerol concentrations induced by norepinephrine was similar during both infusions. Moreover, norepinephrine infusion promoted a similar increase in ethanol ratio, indicating a

Table 2. Area under the curve of extracellular glycerol variation and ethanol ratio

<table>
<thead>
<tr>
<th>Extracellular Glycerol (µM/30 or 60 min)</th>
<th>Ethanol Ratio (%/30 or 60 min)</th>
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<tbody>
<tr>
<td>1st Infusion</td>
<td>2nd Infusion</td>
</tr>
<tr>
<td>30-min Norepinephrine infusion with exercise</td>
<td>4,846 ± 988</td>
</tr>
<tr>
<td>30-min Norepinephrine infusion without exercise</td>
<td>2,615 ± 316</td>
</tr>
<tr>
<td>30-min Dobutamine infusion with exercise</td>
<td>3,408 ± 701</td>
</tr>
<tr>
<td>30-min Terbutaline infusion with exercise</td>
<td>6,097 ± 764</td>
</tr>
<tr>
<td>60-min Norepinephrine infusion separated by 1 h</td>
<td>11,793 ± 2,651</td>
</tr>
<tr>
<td>60-min Epinephrine infusion separated by 1 h</td>
<td>24,452 ± 5,208</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, †P < 0.02, ‡P < 0.01 when compared 2nd infusion vs. 1st infusion.
similar vasoconstriction (Fig. 1B). The calculated AUC values for the variations of glycerol and ethanol ratio were not different (Table 2).

**Effect of Exercise on EC Glycerol Concentrations and Ethanol Ratio Induced by Terbutaline or Dobutamine Infusion in Adipose Tissue**

To further the study of the selective pathways involved in the lipolytic modifications observed with noradrenaline, similar experimental protocols were performed with the use of an infusion of either a selective β1-AR agonist (dobutamine) or a selective β2-AR agonist (terbutaline). Dobutamine infusion induced an increase in glycerol concentration in the EC compartment; the increase was of 286 ± 74 μM after a 30-min infusion (Fig. 2A). After cessation of dobutamine infusion, EC glycerol concentrations fell steadily to reach basal level. The glycerol released in the EC compartment during the second infusion of dobutamine was found to be significantly lower (+127 ± 25 μM) than during the first one (Table 2). Dobutamine infusion promoted discrete vasodilatation (−3.6 ± 2.2% of the ethanol outflow/inflow ratio). The second dobutamine infusion decreased the outflow/inflow ratio similar to the first one (−2.9 ± 2.7%); the AUC values for ethanol ratio variations were not different (Table 2).

Terbutaline infusion induced an increase in glycerol concentration in the EC compartment; the increase was of 336 ± 42 μM after a 30-min infusion (Fig. 2B). After cessation of terbutaline infusion, EC glycerol concentrations fell steadily to reach basal level. During the second terbutaline infusion, the increase of EC glycerol concentration was 234 ± 55 μM after a 30-min infusion. The glycerol released in the EC compartment during the second infusion of terbutaline was found to be significantly lower than during the first one (Table 2). Terbutaline infusion promoted vasodilatation as shown by the observed decrease (−10.5 ± 3.2%) in the ethanol outflow/inflow ratio. The second terbutaline infusion decreased the outflow/inflow ratio similar to the first one (−11.7 ± 2.1%); the AUC values for ethanol ratio variations were not different (Table 2).

**Effect of a Repeated Local Infusion of Norepinephrine and Epinephrine on the Variations of EC Glycerol Concentrations and Ethanol Ratio**

To investigate the putative desensitizing effect of catecholamine on adipose tissue during exercise, two subsequent 1-h norepinephrine or epinephrine infusions (separated by a 1-h interval) were performed, and glycerol responses were evaluated. Norepinephrine infusions induced an increase in glycerol concentration in the EC compartment (Fig. 3A). The EC glycerol concentration increase during the second infusion was significantly lower than the first one (204 ± 35 vs. 270 ± 71 μM after 60-min infusion), and the AUC values for glycerol variations were different (Table 2). Norepinephrine infusion promoted vasoconstriction as shown by the observed increase in the ethanol outflow/inflow ratio (17.0 ± 5.2 and 16.6 ± 6.0% after 60-min infusion). This increase was similar for both infusions, with AUC values for the variations of the ethanol ratio not being different (Table 2).

Epinephrine infusions induced an increase in glycerol concentration in the EC compartment (Fig. 3B). The EC glycerol increase during the second infusion was significantly lower than the first one (395 ± 78 vs. 464 ± 75 μM after 60-min infusion), and the AUC values for glycerol variations were different (Table 2). Epinephrine infusion promoted vasoconstriction as shown by the observed increase in the ethanol outflow/inflow ratio (25.7 ± 8.7 and 20.3 ± 7.6% after 60-min infusion). This increase was similar for both infusions, with AUC values for the variations of the ethanol ratio not being different (Table 2).

**DISCUSSION**

The present study shows for the first time that physiological activation (physical exercise) of the SNS induces desensitization of the adrenergic lipolytic response to local perfusion of catecholamines in subcutaneous adipose tissue in humans. It has been largely demonstrated through in vitro studies on isolated fat cells (14), dialysis studies (4), and binding studies (29) that β1- and β2-AR coexist in human adipose tissue and are both desensitized by adrenergic stimulation.

Our results fit with animal studies showing that sustained adrenergic tone leads to β-AR adipose tissue desensitization (12, 38). In vitro studies have shown that desensitization of the β-adrenergic response occurs in isolated rat adipocytes after acute exposure to isoproterenol (7) and that the activation of cAMP phosphodiesterase is in fact responsible for the desensitization of norepinephrine response as well as for selective β-AR-mediated responses. More recently, studies on isolated human adipocytes have shown similar results (unpublished data), and several lines of evidence support the view that β-AR in human fat cells is desensitized by prior in vitro exposure to β-adrenergic agonists and that the desensitization is associated to a downregulation of β-AR (10, 27). In contrast, Wahrenberg et al. (40) and Savard et al. (33) found that the lipolytic response to catecholamines was increased by 20–35% after an exercise bout on a cycle ergometer. However, in these studies, the adrenergic stimulation was performed by physical exercise just before adipocyte isolation from the adipose tissue. The removal of adipocytes from their actual environment (other cells and plasma factors) before (7) or after (33, 40) the catecholamine stimulation could explain the discrepancy between these studies. Other in vitro studies have found no modification of the isoprenaline or norepinephrine-induced lipolysis in subcutaneous isolated adipocytes after a 100-min exercise in trained or sedentary women (13). On the other hand, the influence of exercise on catecholamine-stimulated lipolysis is known to be location dependent in humans; 30-min submaximal exercise increased β-adrenergic-induced lipolysis of gluteal adipose tissue (40), but the same
exercise did not enhance catecholamine-stimulated lipolysis in abdominal adipocytes (39).

In our in situ study, the exercise blunted the lipolytic response of adipose tissue to norepinephrine as well as to the selective β₁- and β₂-adrenergic agonists. Our results did not fit with those of Wahrenberg et al. (40) and Savard et al. (33). But in their experiments, the removal and the study of the catecholamine-stimulated lipolysis of adipocytes were performed straight after the exercise period, whereas in our work adipose tissue lipolytic response was evaluated in situ after 1-h recovery. We can suggest that, in our study, the length of adipocyte hormonal impregnation could alter their responsiveness to catecholamines. The blunted β-adrenergic lipolytic response appeared to be consecutive to exercise practice, because β-adrenergic stimulation was decreased when norepinephrine was infused 1 h after 60 min of exercise. As a control, the experiment described in Fig. 1B was carried out to show that the responsiveness to norepinephrine was similar when it was infused twice separated by a 3-h interval without exercise. Although lipolytic responses during the first norepinephrine infusion differed between the two experiments (Fig. 1, A and B), this difference did not alter our results. Indeed, we only compared lipolytic responses over the same 1-day experiment because it is well known that adipose tissue responsiveness can differ from day to day for the same subject. Moreover, the probes were not inserted strictly in the same place in the abdominal adipose tissue.

We presume that desensitization resulted from the exposure of adipocytes to increased concentrations of catecholamine during the physical exercise. Nevertheless, other factors could also explain the desensitization. Plasma insulin rebound occurring at the end of exercise could be responsible for an extended antilipolytic effect. Lactate level increase during exercise could also be involved in this phenomenon, although Trudeau et al. (37) have shown recently that lipolytic response in abdominal subcutaneous adipose tissue was not affected by local lactate perfusion. In a previous study, Stich et al. (35) showed that during two identical successive exercise bouts separated by 1 h of recovery, the lipolytic responsiveness was higher during the subsequent exercise bout. However, the increase of plasma epinephrine was dramatically higher, and the insulin level was lowered during the second exercise bout compared with the first one. Therefore, the two-exercise design was not appropriate to reveal possible desensitization. Consequently, we chose to perfuse catecholamine locally before and after the physiological activation. Our previous studies have shown that direct infusions in the subcutaneous adipose tissue of either norepinephrine or epinephrine at 10 μM (30) induced similar lipolytic responses compared with a physiological stimulation by exercise (15).

To assess the effect of catecholamine alone, exercise was replaced by 60-min infusions of epinephrine or norepinephrine (Fig. 3, A and B). Identical patterns to those from exercise stimulation were found, i.e., a reduced lipolytic response during the second infusion period compared with the first one. This result confirms that the effect of exercise on desensitization could result from the exposure of adipocytes to increased concentrations of catecholamine. The fact that the desensitization of human fat cells found in our study was relevant to catecholamine increase during exercise is also supported by the experiments of Stallknecht et al. (34). These authors found that epinephrine infused intravenously led to increased lipolysis in subcutaneous adipose tissue (evaluated by the microdialysis method) and that the lipolytic response was markedly decreased during repeated exposure to epinephrine.

Another study by Klein et al. (25) did not find changes in lipolysis induced by 1-h epinephrine infusion, 90 min after 1 h of strenuous exercise (performed at 70% maximal O₂ consumption on endurance-trained athletes), compared with epinephrine-induced lipolysis without exercise. However, this study investigated lipolysis through the rate of appearance of glycerol in plasma, which reflects whole body lipolysis, and the experiment was performed on endurance-trained athletes. These differences with our study, which used subcutaneous adipose tissue microdialysis in sedentary subjects, could explain the lack of desensitization found by Klein et al. (25). Exercise intensity can also influence adrenergic response to desensitization; indeed, it was shown that cardiovascular β-adrenergic response was blunted after high-, but not low-, intensity prolonged exercise (28).

Moreover, our results showed that the decrease in β-AR-induced lipolysis occurred with β₁-AR (dobutamine) and β₂-AR (terbutaline). These data do not entirely fit with the findings of Arner et al. (2), who demonstrated that a desensitization phenomenon appeared when isoprenaline, epinephrine, or norepinephrine were infused in adipose tissue for 2 h through the microdialysis probe and that this desensitization was found for β₁-AR (dobutamine) but not for β₂-AR (terbutaline). The molecular mechanisms involved in the regulation of β₁- and β₂-AR could explain the observed desensitization of the β₁- and β₂-AR responsiveness. One mechanism involves the phosphorylation of the receptor occupied by β₁- or β₂-agonist, by β-adrenoceptor kinase, and by G protein-receptor kinase. Although most works have been done with the β₂-AR, it is clear that the β₁-AR can also be similarly phosphorylated (36). Another process, involving the consensus sites contained in β₁- and β₂-AR, is the phosphorylation of the receptor by second messenger kinase (PKA, PKC). These mechanisms could also participate in the sequestration of the receptors to an intracellular compartment (36). This last phenomenon (downregulation) leads to a decrease in the density of functional receptors, and it occurs more slowly than the first mechanism.

No data are available concerning the modification of the β-AR number in adipose tissue induced by exercise. However, it has been demonstrated that dynamic exercise induces the translocation of β-AR from intracellular sites to the cell surface in human lymphocytes (18) and in rat myocardium (23). Butler et al. (11)
showed that lymphocyte β-AR responsiveness after exercise was biphasic. Indeed, there was an initial increase of isoprenaline-stimulated cAMP production by lymphocytes immediately after exercise and a decrease thereafter. From these different observations, it is tempting to speculate that this process occurs in fat cells and could also explain the difference found when adipose tissue is investigated in vitro or in our in vivo experiment.

Local microcirculation has been shown to influence glycerol levels in adipose tissue (16, 20). The measurement of ethanol escape through the dialysis probe is a validated nonquantitative method to estimate the changes in vasomotricity induced by adrenergic agonists in adipose tissue (4, 5, 20). In accordance with previous findings, the present study found that norepinephrine perfusion increases the ethanol outflow/inflow ratio, indicating vasosconstriction, and, oppositely, that terbutaline or dobutamine decreases the ethanol outflow/inflow ratio, indicating vasodilatation (5, 30). The present study did not provide evidence that exercise induces a change in adipose tissue blood flow. This suggests that the modifications of interstitial glycerol before and after exercise were influenced, if at all, in the same manner by perfused adrenergic agents. Consequently, the lowered lipolytic response observed after the exercise indicates a true decrease of β-adrenergically mediated lipolysis in adipose tissue.

In conclusion, 1 h after a single bout of physical exercise, the lipolytic response of subcutaneous adipose tissue to catecholamine action is blunted. This decrease accounts for physiological β-AR desensitization. This phenomenon seems to depend on increased adrenergic activity occurring during exercise.

Perspectives

To study subcutaneous adipose tissue β-desensitization in situ without perfusion of any pharmacological agents, we need to find a physiological model inducing two identical successive activations of the SNS. With exercise, it is quite easy to produce two similar activations of the SNS, and plasmatic norepinephrine level suggests that exercise intensity does not change during the two exercise bouts. However, previous data have shown that during a double successive aerobic exercise at 50% \( V_{O_2\text{max}} \) separated by 1-h recovery, plasma epinephrine was significantly higher during the second bout of exercise (35). This might be due to hypoglycemia relative to the first exercise and glycogen needs for the second bout of exercise. Indeed, we have already shown that saccharose ingestion during exercise decreased the plasmatic epinephrine level (15), and it is well known that epinephrine acts as a hyperglycemic hormone during exercise. Therefore, fed compared with fasting subjects could be an interesting physiological model to reveal β-desensitization.

On the other hand, we have also shown (unpublished data) that aerobic exercise at 50% of \( V_{O_2\text{max}} \) performed by endurance-trained subjects did not induce the epinephrine increase during the second bout of exercise, and, consequently, there was no significant difference between the two successive exercise-induced plasma catecholamine levels. Therefore, trained subjects appear to be another interesting physiological model to show β-desensitization.

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